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# A Prospective Study of *N*-Acetyltransferase Genotype, Red Meat Intake, and Risk of Colorectal Cancer[1]

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## ABSTRACT

**Carcinogenic heterocyclic amines are activated by *N*-acetyltransferase (NAT) enzymes, encoded by *NAT1* and *NAT2*, to genotoxic compounds that can form DNA adducts in the colon epithelium. We have examined the relation of polymorphisms in the genes coding for both enzymes to risk of colorectal cancer and the gene-environment interaction with red meat intake among participants in the prospective Physicians' Health Study. Baseline blood samples from 212 men subsequently diagnosed with colorectal cancer during 13 years of follow-up were genotyped, along with 221 controls. *NAT* genotypes were analyzed by a PCR-restriction fragment length polymorphism method. Effect modification of the relation of red meat intake and risk of colorectal cancer by *NAT* genotype was assessed using conditional logistic regression. There was no overall independent association of *NAT* acetylation genotypes and colorectal cancer risk. The relative risks for the rapid acetylation genotype were 0.93 [95% confidence interval (CI), 0.61–1.42] for *NAT1*, 0.80 (95% CI, 0.53–1.19) for *NAT2*, and 0.81 (95% CI, 0.52–1.27) for *NAT1/NAT2* combined. We observed a stronger association of red meat intake with cancer risk among *NAT* rapid acetylators, especially among men 60 years old or older. Among those men who were rapid acetylators for both *NAT1* and *NAT2*, consumption of >1 serving of red meat per day was associated with a relative risk of 5.82 (95% CI, 1.11–30.6) compared with consumption of ≤0.5 serving per day (*P*, trend = 0.02). These prospective data, which need to be confirmed in other studies, suggest that polymorphisms in the *NAT* genes confer differential susceptibility to the effect of red meat consumption on colorectal cancer risk.**

## INTRODUCTION

Germ-line mutations of proto-oncogenes and tumor suppressor genes probably account for <15% of colon cancers (1, 2); the great majority of colorectal cancers appear to be due to noninherited factors (3). Diet has been estimated to account for up to 90% of the international variation in rates of colorectal cancer (4, 5). Red meat intake has been positively associated with colorectal cancer risk (3); however, the mechanisms by which meat consumption may increase risk are not well established. Heterocyclic amines formed during the cooking of meat and other sources of animal protein are potent mutagens and animal carcinogens (6). Heterocyclic amines are initially *N*-oxidized by the polymorphic hepatic CYP1A2[3] enzyme and may then be *O*-acetylated by NAT1 (encoded by *NAT1*) or NAT2 (encoded by *NAT2*) to activated forms that can bind to DNA in the colon epithelium (7, 8). *N*-Acetylation, catalyzed by both NAT1 and NAT2 isoenzymes, may also occur prior to *N*-oxidization, resulting in the less active N-hydroxyarylamide; this conversion is, thus, viewed as a detoxification reaction.

*NAT2* is polymorphic, and the presence of multiple variant alleles divides the population into slow and rapid acetylators and may, thereby, influence individual susceptibility to genotoxic damage due to exposure to heterocyclic amines in cooked meat (9). A higher prevalence of rapid acetylators has been observed among colorectal cancer cases compared with controls (10–12). In a case-control study, Roberts-Thomson *et al.* (13) observed a significant association of high meat intake with risk of colorectal adenoma and carcinoma that was limited to *NAT2* rapid acetylators. Another recent case-control study of colorectal cancer in northeast England (14) also reported an elevated risk associated with frequent fried meat consumption only among *NAT2* rapid acetylators.

*NAT1* was considered monomorphic until the recent discovery of sequence variation in *NAT1* (15, 16) and the existence of rapid/slow alleles (17, 18). Approximately 30–40% of the population with European ancestry carry at least one copy of *NAT1*10* allele, which has been associated with the rapid acetylation phenotype and with significantly higher levels of aromatic amine-DNA adducts in human bladder and colon tissue (18). Data on the association of this *NAT1* polymorphism and colorectal tumorigenesis are sparse and somewhat conflicting. In the only two reported case-control studies, *NAT1* rapid acetylators (defined as carriers of at least one copy of the *NAT1*10* allele) were at significantly higher risk of colorectal cancer (19) but not colorectal adenoma (20); the same finding was observed for rapid acetylators for both *NAT1* and *NAT2* combined. To our knowledge, no study on the interaction of *NAT1* genotype and red meat intake has been reported.

We tested the hypothesis that these polymorphic NAT isoenzymes confer differential susceptibility to colorectal cancer among men in a large prospective study and assessed whether the association of red meat intake with colorectal cancer was modified by acetylation status.

## PARTICIPANTS AND METHODS

The Physicians' Health Study was originally designed as a double-blind trial of aspirin and β-carotene as preventive measures for cardiovascular disease and cancer among 22,071 United States male physicians, ages 40–84 years old, in 1982 (21). About 98% of participants were Caucasian-Americans. Prior to randomization, participants were asked to donate a blood sample, and 14,916 (68%) did so (22). Participants were monitored for incident cancer by regular questionnaires and confirmation from medical records and by searches of the National Death Index. Follow-up for fatal outcomes was 100% and over 99% for nonfatal events. At baseline, participants completed an abbreviated food frequency questionnaire, which inquired about usual consumption of red meat (beef, pork, or lamb as a main dish, as a mixed dish or sandwich, and hot dogs), chicken, and fish. Red meat intake was coded as three categories: ≤0.5 serving per day, >0.5–1 serving per day, and >1 serving per day. An expanded form of this questionnaire has been demonstrated to measure meat intake with acceptable validity among other male health professionals (23). The same questions on meat consumption have been shown to predict risk of colon cancer in women (24) and men (25).

For each confirmed case of colorectal cancer who had provided a blood

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[3] The abbreviations used are: CYP1A2, cytochrome P4501A2; NAT, *N*-acetyltransferase; RR, relative risk; CI, confidence interval.

specimen at baseline, one control was chosen who had not developed cancer at the time the case was diagnosed. For the purposes of another study, nine cases were matched with two controls. Controls were matched by age (±1 year) and smoking (categories were current, past, and never smokers). This analysis includes 212 cases and 221 controls.

There are four relatively common polymorphic alleles for *NAT1*, designated *NAT1*3, NAT1*4, NAT1*10*, and *NAT1*11*. *NAT1*4* is the most common allele. *NAT1*10* is the putative "rapid" allele (18). Genotyping for *NAT1* was carried out using a modification of the procedure of Bell *et al.* (19). Genotyping for *NAT2* was carried out using a modification of the PCR-restriction fragment length polymorphism method of Bell *et al.* (26) that detects the *NAT2* rapid allele (*NAT2*4* [WT]) and the three most common *NAT2* slow acetylator alleles (*NAT2*5A* [M1], *NAT2*6A* [M2], and *NAT2*7A* [M3]) found in Caucasian-Americans. Laboratory personnel were blinded to case-control status.

We calculated RRs and 95% CIs for the association of *NAT* genotypes and consumption of meat with colorectal cancer using conditional logistic regression. The following potential confounding variables were also included in the model: body mass index (<23, 23–25, 25–27, and >27), physical activity (never, less than once a week, and daily), and alcohol intake (same categories as those for physical activity). We assessed effect modification of the relation of red meat intake and risk of colorectal cancer by *NAT* genotype using a likelihood ratio test to compare the goodness of fit of the model with the interaction terms (genotype * red meat), with the reduced model containing indicator variables of the main effects of genotype and exposure (*i.e.*, without interaction terms). The red meat consumption was modeled using the median value of meat intake in each category; *i.e.*, 0.27 for ≤0.5 serving per day, 0.77 for >0.5–1 serving per day, and 1.46 for >1 serving per day. We examined these associations in two strata of age dichotomized by the approximate median of 60 years. All analyses were performed using SAS 6.0 (SAS Institute).

## RESULTS

In our prospective study, the allele frequency of the putative *NAT1* rapid allele (*NAT1*10*) was 23.1% among controls ($n$ = 442 alleles) and 23.3% among cases ($n$ = 424 alleles). The allele frequency in controls was similar to another Caucasian-American population (20.9%; Ref. 20) but higher than a Caucasian-English population (16.1%; Ref. 19). The frequency of the *NAT1* rapid genotype (defined as presence of at least one *NAT1*10* allele) was 43.4% among 212 cases and 44.3% among 221 controls in our prospective study, with an overall RR of 0.93 (95% CI, 0.61–1.42; Table 1). The RR (RR = 0.94; 95% CI, 0.60–1.49) was similar after controlling for potential confounding variables, including red meat and alcohol intake, exercise, and body mass index. When the population was divided into a younger and older group at the median age of 60 years, the association was similar in both younger (<60 years old) and older (≥60 years old) men. Restricting analyses to the 169 cases of colon cancer only (excluding rectal cancer), the RR was 0.92 (95% CI, 0.59–1.45).

The frequency of the three *NAT2* slow acetylator alleles among controls was similar to that observed in a previous study of Caucasian-Americans (26). The frequency of *NAT2* rapid acetylators in cases (38.2%) and controls (43.3%) was not significantly different; the overall RR of rapid *versus* slow acetylation genotype was 0.80 (95% CI, 0.53–1.19; Table 1). Little difference was observed after controlling for potential confounding variables (RR = 0.81; 95% CI, 0.53–1.25). This association was similar in both younger (<60 years old) and older (≥60 years old) men and did not change when the analysis was restricted to colon cancer (RR = 0.77; 95% CI, 0.49–1.21).

We also investigated the association between risk of colorectal cancer and combined *NAT* genotype comparing the *NAT1* rapid/*NAT2* rapid acetylators to the other subjects. The *NAT1* rapid/*NAT2* rapid genotype were slightly underrepresented among cases (22.2%) compared to controls (25.8%; Table 1), but this reduction was not significant (RR = 0.80; 95% CI, 0.49–1.31). The association was not changed when controlled for potential confounding variables, stratified by age, or restricted to colon cancer.

Overall, men consuming meat, on average, more than once a day were at little elevated risk of colorectal cancer (RR = 1.17; 95% CI, 0.68–2.02) compared with men consuming half a daily serving or less (Table 2). We observed a suggestion that the association of red meat intake and colorectal cancer was modified by age ($P$, interaction = 0.03). In analyses restricted to men 60 years of age or older (107 cases and 116 controls), we observed a marginally significant positive association of red meat intake and colorectal cancer (RR = 2.15; 95% CI, 0.95–4.86 for red meat consumption of >1 serving per day compared with ≤0.5 serving per day; $P$, trend = 0.06; Table 3). Neither chicken nor fish consumption was related to colorectal cancer risk, although most men consumed these foods less than once per week.

When we stratified red meat intake by *NAT* genotypes (Table 2), we observed no significant interaction between red meat intake and any of the *NAT* genotypes (all $P \geq 0.16$). Compared to men who were slow acetylators, the association of red meat and colorectal cancer appeared slightly stronger among rapid acetylators for *NAT1* ($P$, trend 0.14 *versus* 0.59) and *NAT2* ($P$, trend 0.35 *versus* 0.96) or both combined ($P$, trend 0.11 *versus* 0.77); however, none of these trends were statistically significant.

Because the association of red meat intake and colorectal cancer risk was stronger among men 60 years of age or older, we examined the interaction of NAT genotype and red meat intake among these older men. When the analysis was restricted to men 60 years old or older (Table 3), the association of red meat intake and colorectal cancer risk among *NAT* rapid acetylators was stronger ($P$, trend ≤ 0.05 for all three genotypes). Among the rapid acetylators for both *NAT1* and *NAT2*, more frequent meat eaters (>1 serving per day) were at significantly higher risk of developing colorectal cancer compared to men who consumed less red meat (≤0.5 serving per day). Among the slow acetylators, this increase in risk was small and nonsignificant. Among older men who were rapid acetylators at both *NAT1* and *NAT2* loci (24%), those consuming >1 serving of red meat per day had an almost 6-fold increase in risk compared to those consuming less than half a serving per day, whereas the increase in risk was only 56% (nonsignificant) among the slow acetylators (Table

Table 1 *Relation of NAT acetylation genotype to colorectal cancer among 212 cases and 221 controls in a prospective case-control study nested in the Physicians' Health Study*

| Gene | Acetylation status | Genotype | Case | | Control | | RR[a] (95% CI) | RR[b] (95% CI) |
|---|---|---|---|---|---|---|---|---|
| | | | *n* | % | *n* | % | | |
| *NAT1* | Rapid | *NAT1*10* allele | 92 | 43.4 | 98 | 44.3 | 0.93 (0.61–1.42) | 0.94 (0.60–1.49) |
| | Slow | Others | 120 | 56.6 | 123 | 55.7 | 1.00 (reference) | 1.00 (reference) |
| *NAT2* | Rapid | *NAT2*4* allele | 81 | 38.2 | 96 | 43.3 | 0.80 (0.53–1.19) | 0.81 (0.53–1.25) |
| | Slow | Others | 131 | 61.8 | 125 | 56.6 | 1.00 (reference) | 1.00 (reference) |
| *NAT1/NAT2* | Rapid | *NAT*10/NAT2*4* | 47 | 22.2 | 57 | 25.8 | 0.81 (0.52–1.27) | 0.78 (0.48–1.27) |
| | Nonrapid | Others | 165 | 77.8 | 164 | 74.2 | 1.00 (reference) | 1.00 (reference) |

[a] Conditional logistic regression model matched on age and smoking history (never, past, or current).
[b] Conditional logistic regression model matched on age and smoking history (never, past, or current) controlled for red meat intake, exercise, alcohol intake, and body mass index.

Table 2 *Relation of red meat intake to colorectal cancer overall and to NAT genotypes*

| Gene | Acetylation | | Daily red meat intake[a] ≤0.5 | >0.5–1 | >1 |
|---|---|---|---|---|---|
| All men | | Case | 62 | 103 | 43 |
| | | Control | 66 | 112 | 39 |
| | | RR | 1.00 | 0.98 | 1.17 |
| | | 95% CI | Reference | 0.64–1.52 | 0.68–2.02 |
| | | | | *P*, trend=0.59 | |
| *NAT1* | Slow | Case | 38 | 55 | 25 |
| | | Control | 31 | 64 | 25 |
| | | RR | 1.00 | 0.69 | 0.82 |
| | | 95% CI | Reference | 0.37–1.26 | 0.40–1.70 |
| | | | | *P*, trend=0.59 | |
| | Rapid (*NAT1*10* allele) | Case | 24 | 48 | 18 |
| | | Control | 35 | 48 | 14 |
| | | RR | 1.00 | 1.50 | 1.71 |
| | | 95% CI | Reference | 0.77–2.93 | 0.76–3.83 |
| | | | | *P*, trend=0.14 | |
| *NAT2* | Slow | Case | 41 | 63 | 25 |
| | | Control | 36 | 67 | 22 |
| | | RR | 1.00 | 0.82 | 1.01 |
| | | 95% CI | Reference | 0.46–1.46 | 0.48–2.13 |
| | | | | *P*, trend=0.96 | |
| | Rapid | Case | 21 | 40 | 18 |
| | | Control | 30 | 45 | 17 |
| | | RR | 1.00 | 1.38 | 1.51 |
| | | 95% CI | Reference | 0.66–2.87 | 0.63–3.63 |
| | | | | *P*, trend=0.35 | |
| *NAT1/NAT2* | Nonrapid | Case | 52 | 78 | 32 |
| | | Control | 45 | 86 | 30 |
| | | RR | 1.00 | 0.82 | 0.93 |
| | | 95% CI | Reference | 0.49–1.33 | 0.49–1.77 |
| | | | | *P*, trend=0.77 | |
| | Rapid | Case | 10 | 25 | 11 |
| | | Control | 21 | 26 | 9 |
| | | RR | 1.00 | 2.13 | 2.35 |
| | | 95% CI | Reference | 0.81–5.65 | 0.77–7.12 |
| | | | | *P*, trend=0.11 | |

[a] *P*, interaction = 0.19 for *NAT1*; 0.56 for *NAT2*; 0.16 for *NAT1/NAT2* combined.

3). In the subgroup of men younger than 60 years, no significant interaction or trend was observed for either genotype (data not shown).

## DISCUSSION

In this prospective study, neither *NAT1* nor *NAT2* acetylation genotype was independently related to overall risk of colorectal cancer. However, consistent with the prior hypothesis that rapid acetylators more efficiently activate heterocyclic amines, we observed a stronger association between red meat intake and colorectal cancer among men with rapid acetylation status at either *NAT* locus or both, especially among the subgroup of men who were 60 years old or older.

Most previous investigations of these associations have been retrospective case-control studies of small size. Ilett *et al.* (Ref. 11; 49 cases), Lang *et al.* (Ref. 10; 43 cases), and Lang *et al.* (Ref. 12; 75 cases of colon polyps and cancer combined) have reported a higher prevalence of *NAT2* rapid acetylators among cases than controls, whereas Rodriguez *et al.* (Ref. 27; 44 cases), Ladero *et al.* (Ref. 28; 109 cases), and Welfare *et al.* (Ref. 14; 174 cases) did not. In a study of 202 colorectal cancer cases and 112 controls, Bell *et al.* (19) observed odds ratios for rapid acetylation of 1.9 (95% CI, 1.2–3.2) for *NAT1* and 1.1 (95% CI, 0.71–1.8) for *NAT2*. However, no significant association with acetylation status at either locus was observed in a study of 441 colorectal adenoma cases and 484 controls (20).

The inconsistent findings of these studies may be due to lack of sufficient exposure to red meat in the study population or selection of a population in which a significant number of participants are <60 years of age. Case-control studies may also be biased, however, if the control series does not represent the population that gave rise to the cases. In a prospective study with high follow-up like this study, recall bias is eliminated because all exposure information is obtained prior to disease; selection bias is also reduced because the source of the population that give rise to the cases is explicitly defined. Most previous reports using cancer as the outcome have studied case series of prevalent colorectal cancers; if acetylation status was associated with survival from colorectal cancer, this would bias the estimate of genotype prevalence among the cases (29). Our prospective data suggest that any independent overall association of colorectal cancer with acetylation status for either *NAT1* or *NAT2* is likely to be weak at most. Although the study population (physicians) may not be representative of the general United States population, it is unlikely that the main effect of these genotypes would be different among Caucasian men with other occupations.

Overall, we observed essentially no association between red meat intake and colorectal cancer, but we did observe a marginally significant positive association among older men (≥60 years old). In the prospective Health Professionals Follow-up Study cohort (25), the positive association of red meat with colorectal cancer was stronger among older men.[4] Similar findings of stronger associations for cruciferous vegetables (30) and protein intake (31) and colorectal cancer in older age groups have also been reported. These results are compatible with the concept that environmental factors act more strongly at older ages, possibly due to a long latent period between exposure and outcome, and the fact that a higher proportion of colorectal cancer at younger age is due to high penetrance mutant alleles in major genes.

When data on gene-environment interactions are presented, two methods of cross-classifying the data are commonly used. One method is to stratify by genotype and examine the exposure-disease

[4] E. Giovannucci, personal communication.

Table 3 *Relation of red meat intake to colorectal cancer risk stratified by NAT genotype among men 60 years old or older*

| Gene | Acetylation | | Daily red meat intake[a] | | |
|---|---|---|---|---|---|
| | | | ≤0.5 | >0.5–1 | >1 |
| Men 60 years or older | | Case | 31 | 50 | 26 |
| | | Control | 44 | 53 | 18 |
| | | OR[b] | 1.00 | 1.36 | 2.15 |
| | | 95% CI | Reference | 0.74–2.52 | 0.95–4.86 |
| | | | | *P*, trend=0.06 | |
| *NAT1* | Slow | Case | 19 | 27 | 14 |
| | | Control | 24 | 29 | 13 |
| | | OR | 1.00 | 1.20 | 1.44 |
| | | 95% CI | Reference | 0.53–2.72 | 0.51–4.06 |
| | | | | *P*, trend=0.53 | |
| | Rapid (*NAT1*10* allele) | Case | 12 | 23 | 12 |
| | | Control | 20 | 24 | 5 |
| | | OR | 1.00 | 1.70 | 3.70 |
| | | 95% CI | Reference | 0.64–4.47 | 1.08–12.7 |
| | | | | *P*, trend=0.03 | |
| *NAT2* | Slow | Case | 20 | 29 | 17 |
| | | Control | 23 | 34 | 13 |
| | | OR | 1.00 | 0.87 | 1.46 |
| | | 95% CI | Reference | 0.38–2.01 | 0.52–4.09 |
| | | | | *P*, trend=0.40 | |
| | Rapid | Case | 11 | 21 | 9 |
| | | Control | 21 | 19 | 5 |
| | | OR | 1.00 | 2.76 | 4.10 |
| | | 95% CI | Reference | 0.94–8.15 | 0.96–17.5 |
| | | | | *P*, trend=0.05 | |
| *NAT1/NAT2* | Nonrapid | Case | 26 | 37 | 19 |
| | | Control | 30 | 40 | 15 |
| | | OR | 1.00 | 1.12 | 1.56 |
| | | 95% CI | Reference | 0.55–2.26 | 0.62–3.93 |
| | | | | *P*, trend=0.39 | |
| | Rapid | Case | 5 | 13 | 7 |
| | | Control | 14 | 13 | 3 |
| | | OR | 1.00 | 3.05 | 5.82 |
| | | 95% CI | Reference | 0.81–11.4 | 1.11–30.6 |
| | | | | *P*, trend=0.02 | |

[a] *P*, interaction = 0.48 for *NAT1*; 0.25 for *NAT2*; 0.25 for *NAT1/NAT2* combined.
[b] OR, odds ratio.

relation separately for each genotype, setting the RR in the lowest category of exposure to 1.0 within each genotype stratum. In our study, among men who were 60 years old or older and who were rapid *NAT* acetylators, consumption of >1 serving of red meat per day was associated with RRs of 3.70 for *NAT1*, 4.10 for *NAT2*, and 5.82 for *NAT1/NAT2*, compared with consumption of ≤0.5 serving per day. Another common presentation is to choose the hypothesized low-risk category of genotype and environmental factor combined as the common reference category and permit the RR of the other genotype in the low-risk category of the environmental factors to depart from 1.0. If we use the low exposure and slow acetylation genotype as the common reference category, the RRs in the rapid acetylator stratum are attenuated. Nevertheless, the association of red meat intake and colorectal cancer was stronger among men who had the rapid acetylation genotype for *NAT1*, *NAT2*, or both (smaller *P*, trend), suggesting that exposure to substrates of NAT isoenzymes in red meat, possibly heterocyclic amines formed during cooking, may play an important role in colorectal tumorigenesis. This interaction is stronger and statistically significant among older men, suggesting that polymorphisms in genes that modify dietary exposures might have more profound impact at older ages.

The roles of *NAT1* and *NAT2* in heterocyclic amine biotransformation are complex. Both enzymes can perform *N*-acetylation (detoxification) and *O*-acetylation (activation), although *in vitro* studies have suggested that the relative capacity of NAT1 and NAT2 to catalyze these reactions varies substantially according to the specific heterocyclic amine substrate (32). In several case-control studies of colorectal cancer and adenomas (12–14), associations with meat intake were limited to or were stronger in *NAT2* rapid acetylators. To our knowledge, no studies have been published on the interaction between *NAT1* genotype and meat intake. The finding that an *NAT1* variant is associated with increased risk of colorectal cancer (19) is supported by a report that the *N*-hydroxy derivative of 2-amino-α-carboline, which predominates in foods at the highest cooking temperatures, is activated by both NAT1 and NAT2 in human liver cytosol (33). These results for the *NAT1*10* allele should be interpreted with caution however, because the functional activity of the various *NAT1* alleles has not been completely defined, and the degree of acetylation activity of the *NAT1*10* allele is still controversial. Bell *et al.* (19) also observed evidence of interaction between the variant allele of *NAT1* and *NAT2*, such that a significantly increased risk of colorectal cancer among subjects with the *NAT1*10* allele was even higher among *NAT2* rapid acetylators. This interaction, however, was not observed by Probst-Hensch *et al.* (20) for colorectal adenomas. In our prospective study, we observed that the association of red meat intake with colorectal cancer was stronger among rapid acetylators at *NAT1* and *NAT2* loci both separately and combined, suggesting that both NAT enzymes play a role in the biotransformation of heterocyclic amine carcinogens.

*NAT1* and *NAT2* are not the only genes involved in heterocyclic amine metabolism. Variability exists in the hepatic *CYP1A2*, the gene that controls the first step in the activation of heterocyclic amines; ~50% of Caucasians are rapid metabolizers (12). The precise genomic alteration responsible for this variation has not been established; however, phenotypic assays to detect the variant metabolizers have been developed (34). In the study of Lang *et al.* (12), 35% of colorectal cancer and polyp cases were both rapid *CYP1A2* metabolizers and rapid *NAT2* acetylators, compared with 16% of controls, suggesting that the combination of the two genotypes is more predictive of risk than either of the two independently. Unfortunately,

phenotyping participants in large prospective studies is problematic; if and when it is possible to genotype *CYP1A2* status will be of great interest. The heterocyclic amine hypothesis also predicts that men who tend to eat their meat more heavily cooked or browned are at higher risk than those who eat less-cooked meat; limited epidemiological data support a possible association with degree of cooking (9). We did not have information on cooking methods in our study so we could not examine this subgroup of meat eaters.

In summary, these data exclude a moderate main effect of rapid acetylation status at *NAT1*, *NAT2*, or both on colorectal cancer risk among men. However, the association of red meat consumption with colorectal cancer was stronger among men with rapid acetylation genotype at either *NAT1* or *NAT2* or at both loci, especially those 60 years old or older. These results, which need to be confirmed in other studies, suggest that heterocyclic amines or other NAT substrates in red meat are important colorectal carcinogens and that cooked red meat consumers may be at higher risk of colorectal cancer if they are *NAT* rapid acetylators.

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